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Development of pyrimidine-based inhibitors of Janus tyrosine kinase 3

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Abstract—A new class of pyrimidine-based Janus tyrosine kinase 3 (JAK3) inhibitors are described. Many of these inhibitors showed low nanomolar activity against JAK3.

Janus tyrosine kinases (JAKs) are a small family of structurally and functionally related non-receptor tyrosine kinases, including JAK1, JAK2, JAK3, and tyrosine kinase 2 (TYK2).[1] JAK-mediated tyrosine phosphorylations of cytokine receptors and signal transducers and activators of transcriptions (STATs) are the important signal transduction pathways used by many cytokines, growth factors, and interferons.[2–4] Unlike the rest of JAK family members that are widely expressed in many mammalian tissues, JAK3 is predominantly located in endoplasmic membranes of hematopoietic cells, and specifically associates with the common cytokine receptor γ chain (γc) which is a shared component of the receptors for IL-2, IL-4, IL-7, IL-9, and IL-15.[5] The pivotal roles in signaling through γc-containing cytokine receptors and unique tissue distribution make JAK3 an ideal biological target to manage the abnormal cytokine activities implicated in many cancer cells and inflammatory lymphocytes.[5]

In comparison to patent claims, the chemistry publications pertaining to JAK3 inhibition are relatively scarce. Stepkowski et al. reported that PNU156804,[6] an analog of antibiotic undecylprodigiosin, effectively blocked JAK3-dependent T-cell proliferation with an IC_{50} of 7.5 μM. This compound showed 2-fold greater specificity versus JAK2-dependent cell proliferation. CP-690,550 is a JAK3-inhibitory small molecule progressed to phase II clinic trials for treatment of acute rejection following kidney transplant surgeries.[7] The oxindole **1** was developed by Aventis from a series designed originally for CDK kinase.[8] In spite of its inherited activities toward CDK kinases, this compound is so far the only reported molecule showing both low nanomolar potency and near 20-fold JAK3/JAK2 selectivity in enzymatic assays (see Fig. 1).

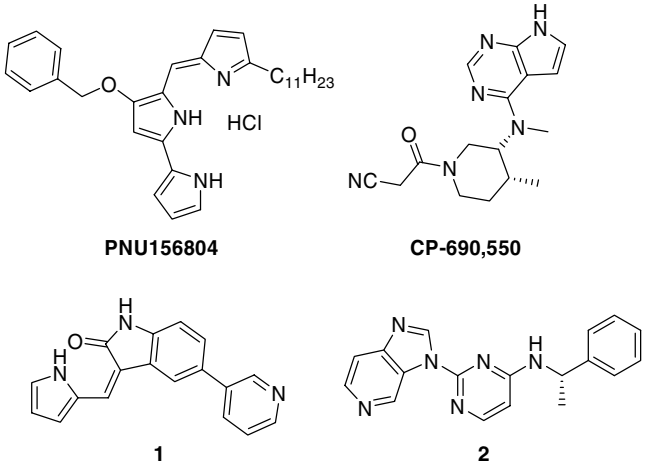

Figure 1. JAK3 inhibitors.

Keywords: Tyrosine kinase inhibitor; JAK3; JAK2; Pyrimidine.
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Our interest in JAK3 aroused from pursuing small molecular therapeutics for autoimmune diseases. Screening the internal kinase compound collections and hit follow-up resulted in 1,4-disubstituted pyrimidines, represented by structure **2**. Further chemistry efforts pursuing this lead produced a novel class of potent and selective JAK3 inhibitors.

Compound **2** and its analogs were easily synthesized with the sequences outlined in Scheme 1, starting from commercially available 4-chloro-2-thiomethyl-pyrimidine **3**. The 'flipped' version (**8**) of **2** was prepared in a similar manner (Scheme 2).

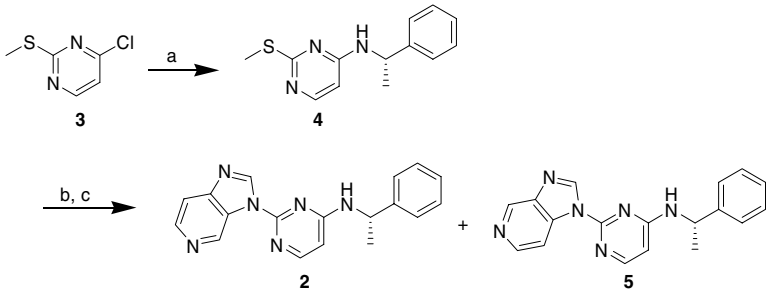
The inhibitory activity of this series against JAK3 and JAK2 was assessed by the kinase-Glo luminescent assay (Table 1) with both CP-690,550 and the oxindole **1** compound as benchmark references.[9] The results suggested that both enzymes prefer the benzimidazole moiety at the 2-position of the pyrimidine core. Switching sequence alignment of amine and benzimidazole usually results in at least sevenfold potency loss (e.g., **8**). The presence of 2-nitrogen atom in the benzimidazole moiety is crucial since its removal resulted in complete loss of activity (e.g., **15**). And 6-azabenzoimidazole regioisomers (e.g., **2**) are usually 20- to 30-fold more potent than the 5-aza regioisomers (e.g., **5**). Methylation on the 2-position of the benzimidazole (e.g., **16**) or the nitrogen of the amine moieties (e.g., **12**) resulted in great reduction of activity. The α-methyl group on the amine side seems also critical since both its removal and restraint caused almost complete loss of potency (e.g., **13** and **14**). In spite of low nanomolar IC_{50} against JAK3, this series in general is more potent toward JAK2. In our hand, the benchmark CP-690,550 showed no selectivity in favor of JAK2 although it has been reported to be 20-fold more selective toward JAK3.[10] And the oxindole **1** is around 16-fold more selective toward JAK3.

Compound **17** was prepared in search for alternative benzimidazole moieties using the sequences outlined in Scheme 3. Equipped with a 6-cyano benzimidazole and a small alkyl amine, this compound maintains the potency of the azabenzimidazole analogs and is 3-fold more selective in favor of JAK3.

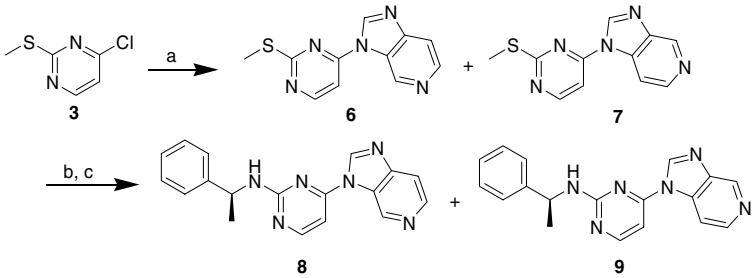
To further augment the JAK3/JAK2 selectivity, structural extension was added to the 6-positions of the pyrimidine core via the sequences outlined in Scheme 4, furnishing the tri-substituted pyrimdine series.

The results (Table 2) unfortunately suggested that neither JAK3 nor JAK2 seems sensitive toward the changes at the 6-position, the modifications nevertheless provide the opportunities for improving the pharmacokinetic profile of this class in future.

To further investigate the in vivo behaviors of the pyrimidine class, selected compounds were tested in TF-1 cell proliferation assays that assess their inhibitory activities toward JAK3 or JAK2-dependent signaling transductions (Table 3).[11] In this measurement,

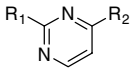


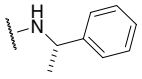
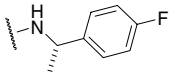
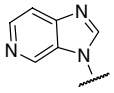
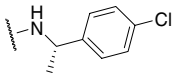
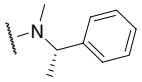
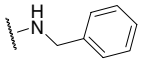
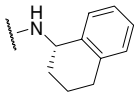
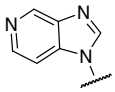
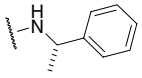
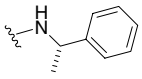
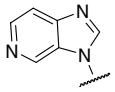
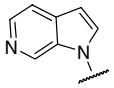
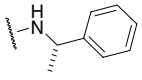
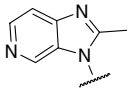
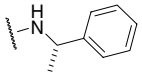
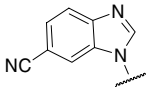
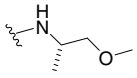
Scheme 1. Reagents and conditions: (a) (*S*)-1-phenylethanamine, $^{i}Pr_2NEt$, Bu_4NI, DMA, microwave (MW), 100 °C, 30 min, 88%; (b) mCPBA, DCM, 0 °C, 10 min; (c) 5-azabenzimidazole, K_2CO_3, DMF, 140 °C, 76%.



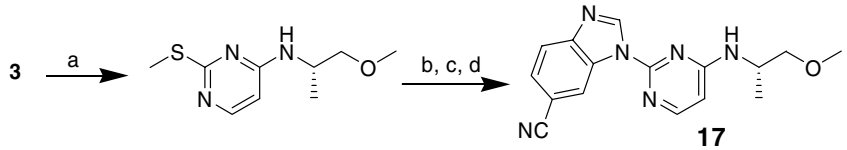
Scheme 2. Reagents and conditions: (a) 5-azabenzimidazole, NaH, Bu_4NI, DMA, rt, 49%. (b) mCPBA, DCM-MeOH, 0 °C, 10 min; (c) (*S*)-α-ethyl benzylamine, $^{i}Pr_2NEt$, DMA, MW, 100 °C, 30 min, 70%.

Table 1. JAK3 and JAK2 kinase data for di-substituted pyrimidine analogs



Compound	R_1	R_2	JAK3 IC_{50}[a] (nM)	JAK2 IC_{50}[a] (nM)
CP-690550	—	—	13[b]	11[b]
1	—	—	38	600
2			21	4
10			92	8
11			1928	51
12			860	153
13			$>10^4$	$>10^4$
14			9097	7218
5			700	51
8			145	49
15			$>10^4$	$>10^4$
16			$>10^4$	$>10^4$
17			45	124

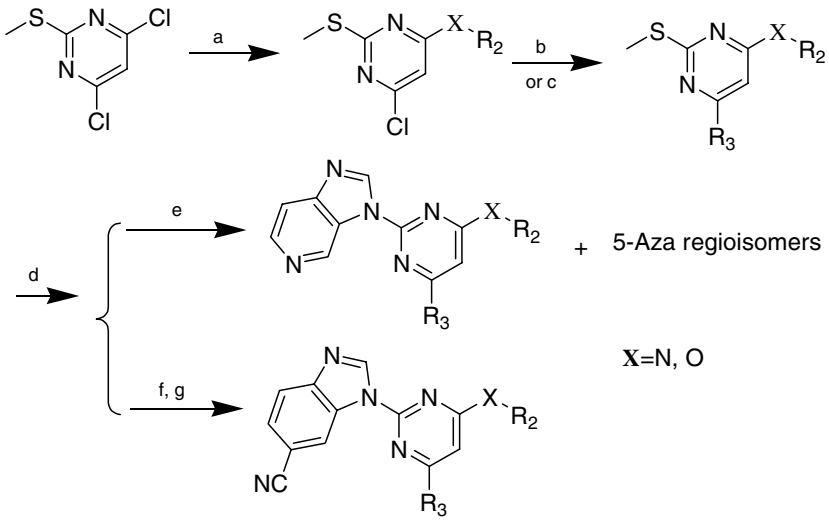
[a] SD for enzyme assays were typically ±25% of the mean or less.
[b] Mean, $n > 10$.



Scheme 3. Reagents and conditions: (a) (*S*)-1-methoxypropan-2-amine, $^{i}Pr_2NEt$, Bu_4NI, DMA, MW, 100 °C, 30 min, 88%; (b) mCPBA, DCM, 0 °C, 10 min; (c) 3-amino-4-nitrobenzonitrile, $K^{t}OBu$, DMF, MW, 100 °C, 15 min, 76%; (d) $SnCl_2{\cdot}2H_2O$, DMF, $(MeO)_3CH$, MW, 120 °C, 30 min, 20%.

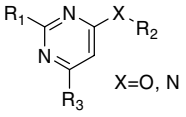
CP-690,550 is about 10-fold more selective toward JAK3, whereas oxindole **1** showed no selectivity. The pyrimidine compound **17** showed 5-fold JAK3/JAK2 selectivity.

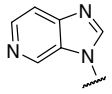
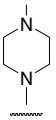
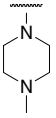
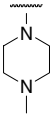
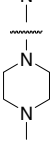
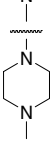
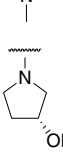
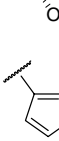
In summary, we have reported a novel series of pyrimidine-based Janus tyrosine kinase 3 inhibitors. Many of the compounds in this series showed strong potency in the JAK3 enzymatic assay below the 100 nM level.



Scheme 4. Reagents and conditions: (a) amine or phenol, $^{i}Pr_2NEt$, Bu_4NI, DMA, MW; (b) $R_2B(OH)_2$, $Pd(Ph_3P)_4$, dioxane, K_2CO_3, MW; (c) amine, $^{i}Pr_2Net$, DMF, MW; (d) mCPBA, DCM, 0 °C, 10 min; (e) 5-azabenzimidazole, K_2CO_3, DMF, 140 °C; (f) 3-amino-4-nitrobenzonitrile, $K^{t}OBu$, DMF, MW, 100 °C; (g) $SnCl_2{\cdot}2H_2O$, DMF, TMOF, MW, 120 °C, 30 min.

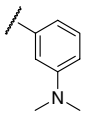
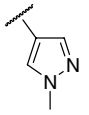
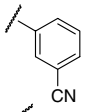
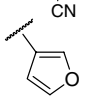
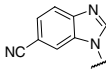
Table 2. JAK3 and JAK2 kinase data for tri-substituted pyrimidine analogs



Compound	R_1	X-R_2	R_3	JAK3 IC_{50}[a] (nM)	JAK2 IC_{50}[a] (nM)
18				19[b]	7[b]
19				61	19
20				27	17
21				120	68
22				131	113
23				331	279
24				180	59
25				60	16

(*continued on next page*)

Table 2 (*continued*)

Compound	R_1	X-R_2	R_3	JAK3 IC_{50}[a] (nM)	JAK2 IC_{50}[a] (nM)
26				62	20
27				470	167
28				49	12
29				245	77
30				42	18
31				167	56
32				165	446
33				31	29
34				83	28
35				51	141

[a] SD for enzyme assays were typically ±25% of the mean or less.
[b] Mean, $n > 10$.

Table 3. Inhibition of proliferation of TF-1 cells, induced by either IL-3 (for JAK2 activation) or IL-4 (for JAK3 activation), by pyrimidine analogs

Compound	Inhibition of IL-4 induced TF-1 cell proliferation IC_{50}[a] (nM)	Inhibition of IL-3 induced TF-1 cell proliferation IC_{50}[a] (nM)
CP-690,550	80[b]	800[b]
1	600	500
10	335	635
11	2010	1400
17	90	440
19	370	580
25	400	610
33	170	600
35	20	30

[a] SD for TF-1 cellular assay were typically ±25% of the mean or less.
[b] Mean, $n > 10$.

And few analogs showed promising selectivity for JAK3 relative to JAK2 in cellular assays.

Acknowledgment

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9. Promega's Kinase-GloTM Luminescent Kinase Assay is a homogeneous non-radioactive method of determining the activity of purified kinases by quantifying the amount of ATP remaining in solution following a kinase reaction. The assay procedure involves addition of a single reagent

(Kinase-Glo® Reagent) directly to a completed kinase reaction. This addition results in the generation of a luminescent signal correlated with the amount of ATP present and inversely proportional to the amount of kinase activity.

10. CP-690,550, reported to possess 20:1 selectivity favoring JAK3 over JAK2 (Ref. 7), showed virtually no selectivity in a separate Kinase Profiler™ Assays (radiometric filter binding assays) run by Upstate Cell Signaling Solutions (JAK3 IC_{50} = 5 nM, JAK2 IC_{50} = 6 nM).
11. All compounds were tested using an assay developed with TF-1 (1, 2) cells (CRL-2003 from ATCC) which proliferate in response to GM-CSF, IL-3 and IL-4. IL-3 (Human recombinant Sigma #I 1646) or IL-4 (Human recombinant. Sigma #I 4269) at 1 nM was used to stimulate the cells. Proliferation of the TF-1 cells was used to measure the responses through JAK2 (IL-3) or JAK3 (IL-4) and the inhibition of such response by specific compounds. Five replicates per compound were seeded at a concentration of 5000 cells/well in a 100 μl volume. Five different concentrations of experimental compounds (5 replicates each) were used to determine their EC_{50}. After 4 days of incubation, cell proliferation was measured during the last 18 h of incubation, using an ELISA (BrdU, chemiluminescence) kit (Roche Diagnostics, Indianapolis, IN). The relative luminescence units (RLU) were measured using a Packard Fusion instrument (Perkin Elmer). The RLU were used to calculate EC_{50}s (Prism4 software).